

ORIGIN OF TASTE BUDS IN THE ORO-PHARYNGEAL CAVITY OF THE CARP.

(*Cyprinus Carpio* Linnaeus).

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According to the doctrine of germ-layer specificity all nervous structures, including taste buds, are derived from the ectodermal layer of the embryo. The mucous membrane lining the digestive tract, including the pharyngeal cavity, is said to originate from the endodermal layer, whereas, that of the oral cavity is generally conceived to arise from ectoderm the presence of which is due to invagination during the formation of the stomodaeum. Most authors agree that the exact limits of ectoderm and endoderm in the oro-pharyngeal cavity cease to be distinguishable after the rupture of the oral plate. In fishes taste buds appear throughout the entire extent of the epithelial lining of this cavity. They occur on the floor, roof, and sides, including the inner surfaces of the gill arches, extending oftentimes even into the oesophagus. In spite of the difficulties in determining the exact boundaries of the two germ layers in this region, the opinion prevails that these structures are derived from ectoderm. In order to account for the presence of these so-called ectodermal derivatives in the pharyngeal cavity, a region generally conceived to be endodermal in derivation, many investigators hold to the opinion that they are derived from ectoderm which has migrated into this region either by way of the oral or the pharyngeal clefts.

Beard ('88, p. 879) claimed that he had evidence to prove that the end organs of taste arise from epiblastic thickenings which have migrated through the gill clefts into the pharyngeal cavity. Fahrenholz ('15) maintained and was supported in his contentions by Jacobshagen ('11, '12) that the oro-pharyngeal cavity in Selachians up to the commencement of the oesophagus is lined with mucous membrane derived from ectoderm. He employed the fact that placoid scales and taste buds occur in this region as evidence to support this contention.

Cook and Neal ('21, p. 48), on the other hand, claimed that in elasmobranchs "the whole pharyngeal cavity is endodermal in its origin and that there is little or no inward migration of the ectoderm into the pharynx." In regard to the origin of taste buds in this region they hold that the endoderm, within which they first make their appearance, is the active layer.

Keibel ('12, p. 183), in discussing this problem, makes the statement that "The majority of the taste buds lie undoubtedly within the entoblastic territory." For this reason he thinks that the doctrine that these structures are ectodermal in origin "is not free from objection."

Many investigators have attempted to account for the origin of taste buds in this region on the basis of their nervous innervation. Although this phase of the problem is not within the scope of the present paper some of the main contentions bearing on this point will be outlined here, since they illustrate, at least, the complexity of the problem as well as the diversity of opinion among investigators.

It has been conclusively established that taste buds in the oro-pharyngeal cavity as well as those on the external surface of the body of fishes are innervated by nerve fibers which belong to the so-called fasciculus communis system. These communis fibers have been traced from their nuclei of origin or termination in the brain, through the roots and ganglia of the cranial nerves, to their peripheral termination. It has been determined that the central termination of these communis fibers is in the vagal and facial lobes of the medulla oblongata. In the family cyprinidæ these lobes are greatly hypertrophied, the latter having fused to form the so-called tuberculum impar. The remarkable enlargement of these lobes is said to be correlated with the abundance of taste buds in the oro-pharyngeal cavity of these fishes.

We are greatly indebted to Professor C. Judson Herrick for much of our present knowledge concerning the peripheral gustatory pathways in fishes. According to this author, whose numerous papers on this subject are well known, taste buds in the oro-pharyngeal cavity are supplied by communis or gustatory fibers from the VII, IX, and X cranial nerves, while those on the outer surface of the body are supplied by the VII nerve. These results have been corroborated by other investigators for various species of fishes.

Allis ('95, '97) claimed that the fasciculus communis system in *Amia* is distributed exclusively to taste buds. Other investigators found that there are two kinds of fibers in this system each of which has a distinct distribution—one to taste or terminal buds and the other to the mucous membrane. The latter are unspecialized visceral sensory fibers ending freely in the mucosa of the digestive tract including the mouth and pharynx.

Johnston ('98, '05, '10) advanced the hypothesis that the communis system is exclusively visceral and hence endodermal, as compared with the general cutaneous and acustico-lateral systems which are related to strictly ectodermal sense organs. Herrick ('98, p. 170) criticised this hypothesis on the grounds that it "seems to lead us into serious difficulties, for, in the first place, the terminal buds of the outer skin, which are very numerous in some fishes and which can hardly be other than ectodermal, are apparently all innervated from the communis system. Again, the taste buds of the mouth of fishes all or nearly all lie in the region of the stomodaeum and are therefore probably of ectodermal origin." Strong ('98 p. 173) likewise offered this criticism, at the same time suggesting that the association of gustatory fibers with visceral fibers "might be accounted for on the supposition that the end bud organs originate on or near entodermal surfaces."

Cole ('98, p. 142) at first considered the fasciculus communis fibers to belong to the visceral system, although he pointed out that it is difficult in the region of the visceral clefts to determine where the somatic region ends and the visceral region begins. In a later paper ('00, p. 320) he seems to think the opposite condition has occurred, that is, "that it was originally a cutaneous system, which has, like the early teeth, invaded the mouth."

In regard to the relationship of the terminal buds in the skin and the taste buds in the oral cavity, Herrick ('99, p. 20) pointed out that "it is generally assumed that these two classes of buds have a common origin, as well as a common structure and innervation." In a later paper ('00, p. 308) he suggested that the terminal buds are probably ectodermal in origin and that "if they arose first as gustatory organs, their migration inwards in the stomodaeum toward the tongue and teeth is intelligible. Whether the others retained this function or became tactile organs, their migration to the exposed surfaces of the body (barblets, fins, etc.) is equally intelligible."

Johnston ('05, '10) entertained an opposite view, namely that the taste organs originated in endodermal territory and secondarily spread to the outer surface of the body. He not only maintained that the evidence is all in favor of the origin of taste buds from endoderm, from the standpoint of nerve distribution, but, also claimed ('10, p. 41) that in teleosts (*Corregonus* and *Catastomus*) taste buds first appear in the pharynx and oesophagus where there seems to be no possibility of origin from any other source than endoderm."

Landacre ('07) in his studies on the time and place of appearance and the direction of spreading of taste buds in *Ameiurus melas* found (1) that they "appear simultaneously in the extreme anterior portion of the oral cavity (ectoderm) and on the endoderm of the first three gill arches," (2) that those of the pharynx spread posteriorly into the oesophagus and that those of the oral cavity spread posteriorly until they reach the pharyngeal group, (3) that no buds spread from the pharyngeal group to the outer surface of the body while the cutaneous buds, which appear later than the oral group, are continuous with the latter just inside the lips. It is evident from these results that this author confirms the occurrence of taste buds in endodermal as well as in ectodermal territory, but disagrees with Johnston's suggestion that buds spread from endodermal into ectodermal territories.

In regard to the possibility that buds in ectodermal territory may actually spread into endodermal territory, this author ('07, p. 47) suggests that this "is probably peculiar to *Ameiurus* and has no bearing whatever on the question as to where taste buds first appeared phylogenetically." However, he expressed the opinion ('07, p. 47) that "the evidence seems to be in favor of Johnston's hypothesis ('05, '06) that buds in primitive forms appear first in endodermic territory, since taste buds are always supplied by communis fibers which are visceral in their relationship as far as their central nuclei are concerned."

Another significant problem in connection with the nervous innervation of taste buds and its possible bearing on their germ layer origin is that in regard to the question as to whether the buds appear fortuitously and independent of their gustatory nerves or whether the nerve fibers take the initiative and stimulate the production of buds in the epithelium. The significance of this problem is apparent as illustrated in the case

of cutaneous and oro-pharyngeal buds which are innervated by communis fibers terminating centrally in a morphological single center and distributed peripherally by the VII nerve.

Olmstead ('20 b) and May ('25) proved by experimental methods that in *Ameiurus* degeneration of the gustatory nerve after sectioning is followed by degeneration of the taste buds and regeneration of the nerve is accompanied by the reappearance of taste buds. These authors hold, therefore, that the presence of the gustatory nerve is the causative factor in the differentiation and transformation of the epithelial cells into taste buds. Professor Landacre ('07) whose investigations on the appearance of taste buds in *Ameiurus* were embryological rather than experimental, made the significant statement in regard to the appearance of taste buds supplied by the VII nerve, that "some of these fibers on reaching the surface produce taste buds, whether in the ectoderm or endoderm."

It is obvious, therefore, that the solution of the problem as to the origin of the taste-buds in the oro-pharyngeal cavity hinges on the derivation of the mucous membrane lining this cavity. In a former paper ('29) the author published the results of a study of an embryological series of carp, ranging in age from the time the eggs were fertilized until fifteen days after hatching—the object being to determine the germ-layer origin of the oro-pharyngeal mucous membrane and its relation to the development of the pharyngeal teeth. In order to determine what germ layers contribute to the formation of the mucous membrane lining this region it was necessary to trace the development of the foregut from the earliest stages of germ-layer differentiation, and, to study the mode of formation of the mouth and gill-slits with the view to determining whether or not ectoderm migrates into the oro-pharyngeal cavity during their formation.

The results of that study presented evidence that the mucous membrane lining the oro-pharyngeal cavity consists of two types of cells each derived from a distinct source, a superficial layer of flattened cells the presence of which is accounted for by the inward migration of the epidermal stratum during development of the mouth and gill-slits, and, a deeper layer of columnar cells. The latter represents the original endodermal layer which was laid down during the formation of the primordial foregut. Furthermore, proof was offered that the enamel

organs of the pharyngeal teeth are formed from the deeper epithelial layer of the pharyngeal mucous membrane and hence are endodermal in origin. Since this was the conclusion arrived at in the case of these structures, which are considered to be ectodermal in origin, the author desired to extend his investigations to other so-called ectodermal derivatives, namely taste buds.

The taste buds in the oro-pharyngeal cavity of the carp (Fig. 1) are essentially similar in structure with those described for other teleosts. They are somewhat pyramidal in shape with the apex projecting a considerable distance beyond the level of the mucous membrane. They consist of a compact group of enormously elongated epithelial cells with greatly attenuated distal extremities and with enlarged basal ends in the region of the nuclei. As this embryological series had been stained with Delafield's haematoxylin and counter stained with eosin, no special staining technique was employed in the study of these buds. Consequently it was impossible to study the structure of the buds in great detail or to determine whether or not so-called sustentacular or basal cells were present. Neither was it possible to determine the relationship of the gustatory nerve endings with the proximal extremities of the taste cells. However, it has been conclusively established by other investigators that the sense cells of the taste bud are in contiguity and not continuous with the gustatory fibers which supply them.

The first appearance of taste buds was observed in a larva 22 hours after hatching (Fig. 2). They were immature, of course, but could easily be distinguished by means of the accumulation of cells into papilla-like structures. They bear a superficial resemblance to the neuromasts, but unlike the latter they arise as evaginations from the basal layer. Furthermore they could not be confused with neuromasts as these do not appear in this region. As can be seen in Figure 2, these immature buds are composed of columnar cells which are continuous with the lowermost epithelial layer of the mucous membrane. The cells are not as yet differentiated into sense cells but are apparently similar to the columnar cells of the basal layer of the mucous membrane with which they are continuous. The superficial flattened epithelial layer of the mucous membrane can be seen to pass uninterruptedly over these groups of proliferating columnar cells.

These primordial taste buds make their appearance simultaneously in the oral and pharyngeal cavities. This agrees with the observations made by Landacre on *Ameiurus* ('07) and is contrary to those made by Johnston ('05 and '10) on *Corregonus* and *Catostomus*. A careful examination of preceding stages failed to reveal the presence of any structures that would likely be immature taste buds.

In Figure 3 is shown a developing taste bud on the roof of the oral cavity of a larva 23 hours after hatching. This bud, with a section of a nerve shown at its base, has evaginated considerably, giving it a more or less oval shape with its cells somewhat radially arranged. By 26 hours after hatching the taste buds in the pharyngeal cavity are not only more numerous but appear to be more highly developed than those in the oral cavity. This observation is in agreement with that of Johnston ('05, '10) for *Corregonus* and *Catostomus* but disagrees with that of Landacre for *Ameiurus* ('07). Figure 4 shows one of these buds on the inner surface of a gill arch. This bud is more highly developed than in figures 2 and 3 with a somewhat oval shape. This increase in the size of the buds seems to be due to the elongation of the cells rather than by their multiplication. The cells in the center of the bud are more elongate becoming gradually shorter until at the margins they pass over continuously into the columnar cells of the mucous membrane. The distal extremities of these cells have not penetrated through the flattened epithelial covering of the bud. Neither have they assumed the attenuated shape of the typical taste cell. However the relationship of these cells to the columnar epithelium of the mucous membrane is still evident. The relation of the superficial flattened epithelial layer of the mucous membrane to the developing buds is quite apparent in these figures. It is evident that it follows their contour but apparently contributes nothing to their formation.

The next stage in the development of the taste buds is represented in Figure 5, which shows their appearance in a larva 28 hours after hatching. The entire bud is still somewhat oval in shape. However, the central cells of the bud, those which are destined to become taste cells, are considerably more elongated than those in the preceding stages. They have begun to become somewhat attenuated at their distal extremities and seem to possess an enlargement near their basal ends, due apparently to the presence of their nuclei. This central core of

elongated sense cells thus assumes a more or less pyriform-shape, whereas the entire bud with its cluster of taste cells and its covering of flattened epithelial cells possesses a somewhat oval-shape. This oval shape is apparently due to the latter layer of cells, which passes over the group of taste cells and covers it in a cap-like manner. The distal or free ends of the taste cells do not appear to penetrate the surface of the mucous membrane, but are apparently covered by the superficial flattened epithelial layer.

Figure 6 shows a taste bud at a later stage of development. This bud is found on the roof of the pharyngeal cavity of a larva 40 hours after hatching. The free extremities of the elongated taste cells have broken through the superficial flattened epithelial covering and end freely above the surface. The superficial epithelial layer, having been broken, now surrounds the attenuated ends of the taste cells thus furnishing the latter with a pore as well as a covering.

The final stage of development is shown in Figure 1, which is a mature taste bud in a larva 42 hours after hatching. The structure of this bud has already been described.

It remains then to interpret its structure in the light of its germ-layer origin. It is evident from the foregoing description of the development of the taste buds in the oro-pharyngeal cavity of the carp that the elongated taste cells are modified or differentiated columnar epithelial cells which are continuous with the lowermost layer of columnar epithelium of the mucous membrane of this region.

It has been conclusively established by other investigators that all taste buds wherever found are derived from the germ-inating or Malphigian layer of the epidermis. Cook and Neal ('21, p. 48) claimed that in *Squalus acanthias* "it is within the endoderm that the taste cells first make their appearance in a 45 mm. embryo by the local thickening of the epidermis and the differentiation of cells of the stratum germinativum." Keibel ('12, p. 184) in his description of the development of taste buds, claimed that "the basal cells of the epithelium lose their usual low cylindrical form and increase in size noticeably."

May ('25, p. 387) stated that when the taste buds degenerate following degeneration of the gustatory nerve "the dermal papillæ are then no longer capped by taste buds, but end among cells which do not differ in appearance from the surrounding epidermal cells." This author claimed that in the process of

formation of taste buds, following regeneration of the gustatory nerve, "it is the ordinary epithelial cells which become transformed into the gustatory cells."

A rather interesting side-light on this problem has been suggested by Botezat and Parker (quoting from Cook and Neal, '21, p. 47) namely, "that these modified epithelial cells to which the name taste buds is given may be primarily secretory, and that the nerves receive their stimulation through the response (secretion) of these cells to the stimulating substances." The latter authors go on to say that "such an explanation would rule out the term 'sense-cell' as applied to the groups of slender cells making up the taste-buds." They add further (p. 52), that if these so-called sense-cells are glandular, the deduction that all nervous receptor cells, including taste cells, are of ectodermal origin is a logical non-sequitur. However, as was suggested by May ('25, p. 404), when the Golgi method is applied to the taste buds and their gustatory nerves the former react in differential staining in the same way as the nerves themselves. It would seem therefore that these modified epithelial cells, which make up the taste buds, assume the characteristics of typical nervous receptors. In spite of this, however, the doctrine that all nervous receptors are ectodermal in origin is not in accordance with the results set forth in the present paper.

It is evident from these results that the superficial flattened epithelial layer of the mucous membrane lining this region contributes nothing to the taste bud other than a covering for the distal or free extremities of the taste cells and that, since it surrounds these extremities, it thus forms a pore. Since the author demonstrated in a previous paper ('29) that the lowermost or columnar layer of the mucous membrane, from which the taste cells are derived, represent the original endodermal layer of the foregut, therefore the conclusion arrived at here is that the taste cells, which form the taste buds, are endodermal in origin.

SUMMARY.

1. The first appearance of taste buds in the oro-pharyngeal cavity of the carp was observed in a larva 22 hours after hatching.
2. These immature buds make their appearance simultaneously in the oral and pharyngeal cavities.

3. By 26 hours after hatching the pharyngeal buds were more numerous and highly developed than the oral buds.

4. Typical mature buds were first observed in the pharynx of a larva 42 hours after hatching.

5. These buds are essentially similar in structure with those described by other investigators for other teleosts. They are composed of elongated taste cells with attenuated extremities which project beyond the superficial flattened epithelium.

6. The superficial flattened epithelium of the oro-pharyngeal mucous membrane contributes nothing to the taste buds other than their covering and furnishes them pores whereby the attenuated extremities of the taste cells reach the surface.

7. Undifferentiated taste cells first make their appearance (22 hrs.) as low cylindrical cells similar to those of the basal epithelium with which they are continuous.

8. These cells gradually elongate and assume the shape and characteristics of typical taste cells.

9. Taste cells are therefore derived from the basal epithelium by modification or differentiation.

10. Since the author demonstrated in a former paper that the basal epithelium of the mucous membrane lining the oropharyngeal cavity is endodermal in origin the conclusion arrived at in the present paper is that the taste buds in this region of the carp are derived from endoderm.

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EXPLANATION OF PLATE.

These Figures are enlargements of microphotographs.
Magnification $\times 1800$.

- FIG. 1. A mature taste bud on the inner surface of a gill arch in a larva 42 hours after hatching.
- FIG. 2. The first primordial taste bud to be observed in the series. Found on the roof of the pharyngeal cavity in a larva 22 hours after hatching.
- FIG. 3. A developing taste bud found on the roof of the oral cavity in a larva 23 hours after hatching.
- FIG. 4. An immature taste bud on the inner surface of a gill arch in a larva 26 hours after hatching.
- FIG. 5. A taste bud found on the roof of the pharyngeal cavity in a larva 28 hours after hatching.
- FIG. 6. An almost mature taste bud on the roof of the pharyngeal cavity in a larva 40 hours after hatching.

NOTE.—In all these figures the superficial flattened epithelial layer of the mucous membrane lining the oro-pharyngeal cavity is comparatively easily distinguishable from the basal columnar layer. Nuclei of the cells in the former layer are shown in most of the figures, especially where this layer covers and follows the contour of the taste buds. The relation of these cells to the taste buds is quite evident in Figure 6.

